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ASSESSMENT OF THE INFLUENCE OF KETOCONAZOLE ON MEMORY RETRIEVAL AMONG ADULT WISTAR RATS.

[1] Nyaribo Cyprian Mose [*2]Nyabayo Josephat Maniga, [3]Kerubo Eunice Bogecho [4]Marera Dominic Oduor. [5]Nyanchoka Albert Onchweri, [6]Miruka Conrald Ondieki.

[1]School of Pharmacy and Health Sciences, United States International University – Africa, Nairobi, Kenya.
[2]Department of Microbiology, [3]Department of Public Health, [5]School of Pharmacy, [6]Department of Biochemistry, Kampala International University, Western Campus, Ishaka, Bushenyi Uganda.
[4]Department of Human Anatomy, Maseno University, Maseno, Kenya.
Email:nyacyprian2@gmail.com

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Abstract

The aim of the study is to investigate the influence of ketoconazole on memory retrieval among adult wister rats in relation to age and gender. Anxiety disorders are of concern and definite treatment is wanting in current medicine. Ketoconazole, a known glucocorticoid synthesis inhibitor was used in this study to reduce memory retrieval in rats which were subjected to a stressful and life threatening water maze training. We wanted to evaluate the time difference after ketoconazole treatment and possible hippocampus neuronal histological changes in the treated animals.At the end of this research study which lasted for eight (8) months, remarkable and appreciable results were found. On the average duration taken by the animals from the four different starting points of swimming trials, the differences between the values of escape latency before and after treatment were found not to be statistically significant ($p>0.05$). However, there was a possibility of handedness among animals as seen while turning right to the platform.We concluded that, the administration of ketoconazole did not exhibit significant inhibition of glucocorticoid synthesis since the memory retrieval and hippocampus neuronal morphology between the treated and untreated animals did not show significant statistical difference.

Keywords: *Glucocorticoid, Ketoconazole, Hippocampus, Learning, Memory, Maze.*

INTRODUCTION

Globally there have been a number of neuronal degenerative conditions which pose a threat to people because there regimen has not been cleared found. Ageing has also been associated with a number of conditions which present with poor learning and memory (Burke et al., 2006). It is known that glucocorticoid hormone plays a major role in learning and memory retrieval. These neuronal degenerative conditions are also noted in Africa especially in east Africa.

Glucocorticoids being a class of steroid hormones do bind to the glucocorticoid receptor, which is present in almost every vertebrate animal cell. This hormone enhance learning and memory retrieval in vertebrates and its level change in rats secondary to administration of its inhibitor like ketoconazole is intresting. (Buchanan *et al*., 2001).

The effect of glucocorticoids variation depends on the level of the hormone and the emotional status of the memory. There is evidence that the glucocorticoid-induced retrieval impairment occurs specifically for emotionally arousing information or emotionally arousing testing situations like stress (Dahl R.E 2001)

Glucocorticoids influence memory processing (encoding, consolidation and retrieval) in all stages (de Quiervain *et al*., 2009). The effect varies depending on the level of the hormone and on how emotional the memory is. For the effect on encoding of new information, in particular, an inverted U shaped function has been proposed, with moderate glucocorticoid concentration enhancing this process, but very low and very high concentrations exerting a diminishing effect, (Roozendaal *et al*., 2002). There is evidence that the glucocorticoid-induced retrieval impairment occurs specifically for emotionally arousing information (Buchanan *et al*., 2001) or emotionally arousing testing situations.

Cortisol levels are naturally not only increased by stress, but also due to circadian rhythm and an additional response to morning awakening. Cortisol is also distinctly increased due to stress levels in the morning hours, (Buchanan *et al*., 2001). Memory retrieval indeed seems to be less efficient in the morning hours compared to afternoon or evening hours, when cortisol is low (Célérier *et al*., 2004).

It is also documented that β-adrenoreceptor activation in the hippocampus and the basolateral complex of the amygdala (BLA) is implicated in the impairing effects of glucocorticoids on memory retrieval (Roosendaal *et al*., 2004). Reports also indicate that glucocorticoids impair retrieval of memory by facilitating noradrenergic mechanisms in the hippocampus and additionally, that norepinephrine-mediated basolateral complex of the amygdala (BLA) activity is critical in enabling hippocampal glucocorticoid effects on memory retrieval (Roosendaal *et al*., 2004).

MATERIALS AND METHODS

Animals Handling

Thirty young adult wister rats weighing 100-220 g at the time of the beginning of the experiment were used. The animals were housed in a room temperature and maintained on a standard 12 hr light/dark cycle (lights on from 7:00 am-7:00 pm) in the Department of Human Anatomy, Kampala International University. Food and water were administered *ad libitum*. Water maze training and testing was performed during the light phase of the cycle between 9:00 am to 3:00 pm. The Moris water maze measuring 1.5 m in diameter, and 0.60 m in height was filled with milky water (25^0C) to a depth of 30 cm. A 10 cm diameter circular platform was placed at a fixed location, 25 cm away from the edge of the pool. The platform was submerged 2.0 cm below the water surface so that it could not be visible to the rats.

For spatial training the rats were given four swimming trials on each daily session for three consecutive days. This relative small number of trials was chosen such that retention performance of

control of animal could be moderate and drug administration could either enhance or impair performance.

Before the first training trial, the rats were placed directly on the submerged platform for 30 seconds. On each of the trial (swimming) the rat were placed into the tank at one of the four designated starting points in a random order and allowed to find and escape onto the platform. For the animals which could fails to find the platform within 60 seconds, they were manually being guided to the platform. After mounting the platform, the rats were allowed to remain there for 15 seconds and were then being placed into a holding cage for 25 seconds until the start of the next trial. The time each rat spent to reach the platform was recorded as escape latency. Retention of the spatial training was assessed 24hr after the last training session

This study was carried out at the department of Human Anatomy, Kampala International University Uganda. It was conducted in the month of January, 2014.

Drug Administration

Ketoconazole dissolved in water for injection was administered orally. Control animals received the vehicle (water for injection). The drug was given twelve hours after the water maze training and testing at dosing intervals eight hours for one day. Six different groups of five animals grouped as, untreated female, untreated male, minimum dose male, minimum dose female, maximum dose male and maximum dose female. The minimum dose was 3.3 mg/kg body weight and the maximum was 6.6 mg/kg body weight.

Hippocampus Histological Analysis:

At the end of the training testing and treatment the rats were sacrificed under deep anaesthesia by diethyl ether in order to avoid ain infliction. The brain was surgically removed and immersed in fresh 4% formaldehyde, at least 24 hr before sectioning. The brain was transferred to a 20% sucrose solution in a saline for cryoprotection. The tissue was then processed and stained by use of Hemotoxylene and Eosin stains to assess the effect of ketoconazole on the hippocampal neuronal dendrites. The neuronal dendritic variations of the hippocampus of the treated and the control tissues were analyzed histologically under high magnification to assess the differences in the dendritic population.

RESULTS

Weight and sex distribution

A total of 30 Wister rats were used in this experiment, the animals were in six groups of five animals each. Four groups were treated with ketoconazole and the two groups were the control in the analysis of the memory retrieval and hippocampal histological dendritic changes.

After grouping the animals into different weight and sex, it was noted that the male rats weighing between 101-110 were 1(6.7%) while the female were 0(0%), the rats which were weighing between 111-120 represented male 2(13.3%) and female were 2(13.3%). The animals with the weight range of 121-130 were male 2(13.3%) and the female were 1(6.7%), in the category of body weight of 131-141were male 6(40%) while the female were 2(13.3%). The animals of weight range of 141-150 were male, 1(6.7%) and female were 1(6.7%), in the range of 151-160, the male were 1(6.7%) and the female were 1(6.7%). Those animals with the weight range of 161-170 were male 0(0%) and female 2(13.3%), those in the range of 171-180 were male 0(0%) and female 2(13.3%) those in the range of 181-190 were, male 1(6.7%) and female 1(6.7%). Those in the range of 191-200 were male 090%) and female 2(13.3%), and finally those in the range of 201-210 were male, 1(6.7%) and female were 1(6.7%).

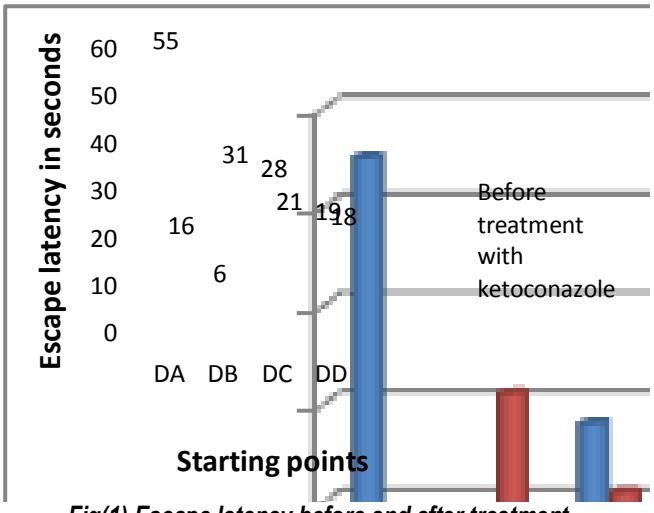
The mean body weight of the animals used was 139.2 grams. The total number of males and females were 50% (15/30) and 50% (15/30) respectively as shown in table 1 below.

Table(1)Weight and sex distribution

SEX	WEIGHT RANGE (GRAMS)											Total
	101-110	111-120	121-130	131-140	141-150	151-160	161-170	171-180	181-190	191-200	201-210	
Male	1	2	2	6	1	1	0	0	1	0	1	15
%	6.7	13.3	13.3	40.0	6.7	6.7	.0	.0	6.7	.0	6.7	100.0
Female	0	2	1	2	1	1	2	2	1	2	1	15
%	.0	13.3	6.7	13.3	6.7	6.7	13.3	13.3	6.7	13.3	6.7	100.0
Total	1	4	3	8	2	2	2	2	2	2	2	30
%	3.3	13.3 %	10.0	26.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	100.0

Escape latency before and after treatment

From a sample size of 30, the range statistics from different starting points were DA(55), DB(6), DC(28), DD(19) with a total of 65 before treatment and DA(16), DB(31) DC(21) DD(18) after treatment. The minimum statistics were DA (1), DB (2), DC (1), and DD (10) with a total of 64 before treatment and DA (16), DB (31), DC (21), and DD (18) with a total of 52 after treatment. The maximum statistics were DA (56), DB (8), DC (29), and DD (20) with a total of 70 before treatment and DA (18), DB (33), DC (22), and DD (19) with a total of 61 after treatment. The statistical mean for escape latency was DA (6.5), DB (3.6), DC (4.3), and DD (3.4) with a total of 17.9 before treatment and DA (6.0), DB (7.3), DC (4.2), and DD (3.7) with a total of 21.3 after treatment as indicated below.

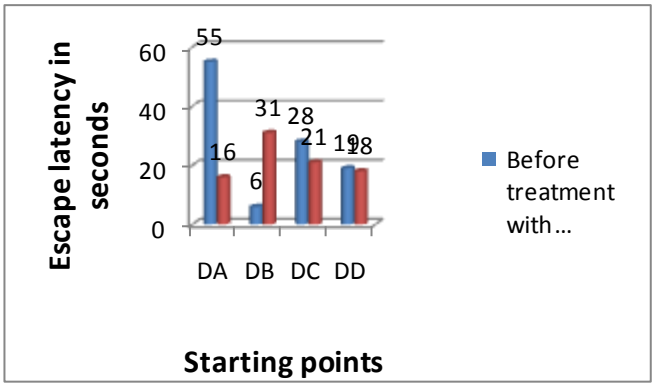


Fig(1) Escape latency before and after treatment

Effects of the glucocorticoid synthesis inhibitor, ketoconazole, on memory retrieval among trained adult wistar rats

From a sample size of 30, the range statistics from different starting points which were labelled designation(D) A, B, C and D were DA(55), DB(6), DC(28), DD(19) with a total of 108 seconds before treatment and DA(16), DB(31) DC(21) DD(18) with a total of

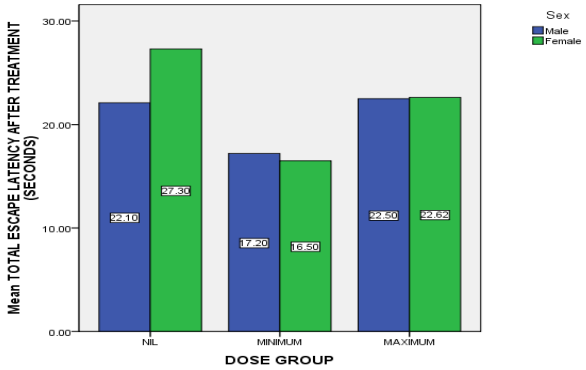
86 seconds after treatment. The minimum statistics were DA (1), DB (2), DC (1), and DD (10) with a total of 14 seconds before treatment and DA (16), DB (31), DC (21), and DD (18) with a total of 86 seconds after treatment. The maximum statistics were DA (56), DB (8), DC (29), and DD (20) with a total of 103 seconds before treatment and DA (18), DB (33), DC (22), and DD (19) with a total of 92 seconds after treatment. The statistical mean for escape latency was DA (6.5), DB (3.6), DC (4.3), and DD (3.4) with a total of 17.9 before treatment and DA (6.0), DB (7.3), DC (4.2), and DD (3.7) with a total of 21.3 after treatment.



Fig(2) Escape latency before and after treatment

Effects of different ketoconazole doses on memory retrieval

When different doses of ketoconazole which represented nil, minimum and maximum were administered to different groups of rats, there was no specific trend one could observe in results. In some cases the escape latency before treatment appeared to be higher than after administration of minimum and maximum doses of ketoconazole. Even when the average mean escape latency before and after treatment with the different ketoconazole doses was considered, the difference was found not be statistically significant as indicated in the figure below.



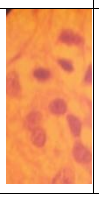
Fig(3) Effects of different ketoconazole doses on memory retrieval

Effects on hippocampal neuronal dendrites

For both control and test animals the three distinct layers (strata) of the hippocampus were visible in both the low and high magnification slides. These were; a polymorphic granulosum layer containing many nerve fibers and small cell bodies of interneurons, the middle pyramidal cell layer containing hippocampal pyramidal cells, and finally the molecular layer containing dendrites of the pyramidal cells.

Examination of the three layers which form the hippocampus, that is stratum granulosum, stratum pyramidale and stratum moleculare, at low magnification revealed no structural differences in dendritic growth between tissues from control and test the animals. In the high magnification slides, multiple neurons could be seen in all the slides with numerous dendrites but these still had a similar appearance in terms of dendritic population in test animals as it was in the control animals. No major structural differences were observed even with varying doses of ketoconazole which was suggestive of the no morphological changes in the neurons of the hippocampus of all the six groups of animals as indicated in the table below.

Table (2) Effects on hippocampal neuronal dendrites

	Control animals		Minimum dose		Maximum dose	
	Low magnification *40	High magnification *1000	Low magnification*40	High magnification*1000	Low magnification*40	High magnification*1000
Female						
Male						

DISCUSSION

Weight and sex of the animals

Given that the animal's body weights were used as a parameter to determine their age, it was important to determine their weight and cluster them so that their ability to swim would be evaluated in relation to their weight. It was also important to classify their weights in reference to sex because it is known that the male rats do have more weight than the female rats. After grouping the animals into different weights and sex, it was noted that there was no much discrepancy in weight in relation to sex.

Escape latency before and after treatment

From the four different starting points which were positioned as: DA which was positioned very close to the escape plat form and directly behind it, DB was positioned obliquely on the left side of the escape plat form, the DC starting point was positioned directly opposite the plat form, and finally the DD plat form was positioned obliquely on the right side of the platform. The outcome indicates that the escape latency after treatment took more time than before treatment; this could be secondary to the administration of ketoconazole a glucocorticoid synthesis inhibitor which lowers memory retrieval as it was seen in the literature. However, the differences in escape latency were found not to be statistically significant.

Effects of body weight on learning and memory retrieval

An interesting finding on the average duration taken by the animals from the four different starting points which were positioned as follows, the DA starting point was positioned very close to the escape plat form and directly behind it, DB was positioned obliquely on the left side of the escape plat form, the DC starting point was positioned directly opposite the plat form, and finally the DD plat form was positioned obliquely on the right side of the plat form.

On the correlation of weight with the dependent variable of

escape latency before treatment at DA, DB, DC and DD, it was found that the heavy animals had larger escape latency. However, the difference between treated and untreated animals was found not to be statistically significant ($p>0.05$) for starting positions of DA, DB, DC and DD. With regard to weight the weight of different animals did not show much discrepancy in the results because there was even age distribution for all sexes in all the study groups; however the statistical mean for escape latency indicates that the escape latency before and after treatment for those animals which weighed more took more time than those weighed less.

Before the administration of ketoconazole the animals with more weight were found to have more escape latency as compared to those with less weight. Given that the weight of the animals were reflective of their age, (Buchanan *et al*., 2001) this could be explained by the fact that the aged animals have reduced muscle activity as compared with the young and active animals as it was seen in the literature. The alternative explanation could be that the learning and memory retrieval ability reduces with age.

Effect of sex on escape latency

On the effect of sex on the total escape latency before and after treatment, it was found that females had a lager escape latency than the males. Again the differences between the sexes were found not to be statistically significant ($p>0.05$) for starting positions DA, DB, DC and DD. Also, it was noted that despite the mean time taken by the animals before treatment (escape latency before treatment) being higher than the escape latency after treatment, there was a discrepancy with the escape latency from the four different starting points; for all the tests, including the controls. The female animals took more time in terms of escape latency before and after treatment as compared to the male animals. Given that all the animals were housed together before and after the training and testing period then it could be possible that some of the female animals were pregnant during this period hence a difference in escape latency time given that pregnancy could directly affect the physical activities of the pregnant animals.

Effects of the glucocorticoid synthesis inhibitor, ketoconazole, on memory retrieval

From the paired t-tests, the animals took less time as escape latency from designation DA, DC and DD after treatment as compared to before treatment. The escape latency before and after treatment at DA, DC, and DD was, however found not to be statistically insignificant ($p>0.05$). This is against the expected results because the glucocorticoid synthesis inhibitor ketoconazole was administered and the escape latency was expected to take more time than before treatment. Schäcke *et al*., (2005) also noted that administration of glucocorticoid synthesis inhibitor, ketoconazole in patients with depression and emotional conditions did not show reliable results.

However, in case of the escape latency before and after treatment at DB, it was found to be significant ($p<0.05$). At DB, the animals took more time to find the platform after treatment than it took before treatment, the time difference could be explained by the administration of ketoconazole which could have reduced the memory retrieval similar to what was reported by Malison *et al*., (1999). However, there was a striking difference between the escape latency registered when the animals had to make a right-turn, on one hand, and the results got for straight towards the safety platform.

It was interesting that the animals took relatively short escape latency from DA, DC, and DD but longer time from DB. It was expected that the animals would take almost similar time between DB and DD given that they were all positioned obliquely and within the same distance from the plat form. An element of handedness during swimming to the safe platform could have played an important role in the trend of delayed time seen in most of the animals to find the platform from DB given that all other parameters like extra maze cues and distance remained constant. Handedness in negotiation towards the platform during navigation (swimming) could explain the discrepancy given that from DB the animals were negotiating towards the right and in DD they were negotiating towards the left side in order to find the plat form, hence the variations in time difference from the two diagonal starting points that is DB and DD. This compels one to think that the rats could be exhibiting handedness with regard to the escape latency from point DB, where the animals had to make a right-turn which demands for increased activity of the left hand and a more reduced activity of the right hand.

Effects of different ketoconazole doses on memory retrieval

From the different doses of ketoconazole which represented nil, minimum and maximum which were administered to different groups of rats, trend in the results does not reflect what was documented by Malison *et al*., 1999. According to Wolkowitz *et al*., 1999, animals with higher doses of ketoconazole are expected to take more time to find the platform than those without treatment but the trend in the results does not indicate this expectation. This can be taken to mean that ketoconazole administration did not have a significant effect glucocorticoid levels or it could be that there other factors at play which need to be investigated further.

Histological analysis of hippocampus

The slides of the control animals show features similar to those of the normal hippocampus with its distinct layers as it was seen in the literature review. The examination of the low magnification of the three layers in the animals which were administered low doses of ketoconazole shows similarity with those of the control group in terms of the width of the layers which form the hippocampus that is stratum granulosum, stratum pyramidale and stratum moleculare. In the high magnification slide, multiple neurons are seen with numerous dendrites which have a similar appearance in terms of dendritic population as it was in the control animals.

The comparison of the dendritic population between the slides of animals which were administered minimum doses of ketoconazole and those which were administered maximum doses of ketoconazole shows similarity I terms of distribution and population. The similarity in the three sets of slides with different doses of ketoconazole is suggestive of the no morphological changes in the neurons of the hippocampus. Another finding was on the neuronal histological picture for both the control and the treated animals which indicated no clear discrepancy in terms of the morphological appearances of the strata which form the hippocampus. The layers contain the same width and population of the dendrites as those of the slides of the control animals. This could be attributed to the fact that the ketoconazole treatment of the animals did not change the memory retrieval pattern and hence the morphological appearance of the dendrites of the neurons.

CONCLUSION

From the results of the study, the following conclusions can be made;

There was significant correlation between learning and memory retrieval and body weight. Since body weight was reflective of animal age, it's conclusive that the older animals took a bit longer to learn but there was no significance in memory retrieval.

There was no significant correlation between learning and memory retrieval, and sex although some variation was observed. This explains that all sexes have the same ability of learning and memory retrieval.

Ketoconazole treatment does not have significant influence on memory retrieval, however higher (maximum) doses of ketoconazole showed more significance (Figure 2), which means Ketoconazole should not be considered a suitable regimen for the treatment of anxiety disorders as much as it is a glucocorticoid synthesis inhibitor.

Histological assessment shows that ketoconazole does not cause any dendritic changes. This is an indicator that it did not inhibit the synthesis of glucocorticoid hormone, Figure

The study results also show that animals might exhibit handedness, this was noted as most animals were taking longer time to turn to the submerged platform from starting point DD which was to the left from as compared to starting point DB which was on the right side, this is supported by the fact that there is no clear information on the possibility of handedness in animals.

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